Kidney International, Vol. 68 (2005), pp. 1223–1229

In uremia, plasma levels of anti-protein C and anti-protein S antibodies are associated with thrombosis

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In uremia, plasma levels of anti-protein C and anti-protein S antibodies are associated with thrombosis.

Background. Vascular access thrombosis is an important cause of morbidity in patients with end-stage renal failure on maintenance hemodialysis (MHD). However, little is known about its risk factors. The present study was undertaken to evaluate the role of coagulation factors, fibrinolytic factors, and antiphospholipid antibodies (aPL). In particular, we have evaluated the role of anti-protein C and anti-protein S antibodies in patients on MHD with and without thrombosis because no data are available in the literature.

Methods. The study group comprised 30 patients with thrombotic complications (TC), 40 patients matched for age, sex, and dialytic age with no thrombotic complications (NTC) and 400 controls. We have measured: anti-protein C antibodies, anti-protein S antibodies, anticardiolipin antibodies (ACA), anti-β2-glycoprotein antibodies (β2-GPI), and anti-prothrombin antibodies (aPT), along with prothrombin time, fibrinogen, plasminogen, protein C, protein S, anti-thrombin III, APC-resistance test, D-dimer, tissue-type plasminogen's activator, plasminogen activator inhibitor-1 (PAI-1), prothrombin fragment 1+2, factors of the intrinsic and extrinsic pathway, C-reactive protein, and homocysteine.

Results. There were no significant differences between groups for prothrombin time, fibrinogen, plasminogen, protein C, protein S, anti-thrombin III, activated protein C (APC) resistance, D-dimer, tPA, C-reactive protein, Factors II, X, and VII. The anti-β2-GP1 and aPT were elevated in both TC and NTC patients, compared to the control group. Significant differences between TC and NTC groups were found for anti-protein C and anti-protein S antibodies, ACA-IgM, PAI-1, Factor VIII, prothrombin fragments 1+2, and homocysteine.

Conclusion. The most novel finding was a significant elevation of anti-protein C antibodies and anti-protein S antibodies in the TC group (i.e., in patients on MHD with thrombosis of vascular access). It indicates that other pathogenetic mechanisms in addition to endothelial damage may cause hypercoagulability in uremia.

Key words: hemostasis, dialysis, thrombosis, endothelial damage, anti-protein C antibody.

Received for publication April 26, 2004
and in revised form December 18, 2004 and March 10, 2005
Accepted for publication April 14, 2005

Patients with chronic renal failure on maintenance hemodialysis (MHD) frequently undergo thrombotic events that occur at the site of vascular access, as well as in the coronary, cerebral, and retinal arteries [1–6]. The pathogenetic mechanism is still unknown. Available data have disclosed a state of hypofibrinolysis characterized by elevated plasma levels of the inhibitor of tissue activator of plasminogen (PAI-1) in uremia. Data on other coagulation factors are still being debated [7–11]. Some studies have pointed to increased levels of Factor VII and Factor VIII [12]; other studies have disclosed a reduction in the concentration of activated Factor XII. Reduced plasma concentrations of protein C (PC), anti-thrombin III (AT III), and plasminogen have been described, but the data need further confirmation [13].

Progress in the clinical pathology of thrombotic events has focused on antiphospholipid (aPL) antibodies (Abs), a heterogeneous family present in systemic lupus erythematosus where thrombotic phenomena occur frequently. These antibodies are also found in patients without lupus. They have been considered to be primers for the hypercoagulative state, although available literature does not provide a clear-cut mechanism. In fact, aPL have been found in a variety of clinical conditions, such as venous and arterial thrombosis, thrombocytopenia, cerebrovascular ischemic diseases, recurrent abortion, and cutaneous lesions [14]. It is now possible to measure lupus anticoagulant antibodies (LAC), anticardiolipin antibodies (ACA), antibodies to β2-glycoprotein type I (β2-GPI), anti-prothrombin antibodies (aPT) [15–19], anti-protein C antibodies, and anti-protein S antibodies.

The presence of aPL might induce a prothrombotic state by blocking protein C and protein S, potent natural anticoagulants [15]. Plasma from patients with elevated aPL is resistant to activated protein C and, therefore, has a tendency to formation of thrombin. These antibodies could bind with negatively charged target molecules represented by cofactors to Factors V and VIII, thus causing continuously elevated levels of plasma thrombin. Current

Table 1. Characteristics of patients with thrombotic complications (TC) and patients with no thrombotic complications (NTC)

	TC	NTC	*P* value[a]
Number of patients *N*	30	40	0.86
M/F	15/15	22/18	0.86
Age *years*	54 ± 4	53 ± 5	0.37
Dialytic age *months* (mean)	30 ± 0.5	30 ± 0.8	1.0
Hemoglobin *g/dL*	11.8 ± 0.5	12.0 ± 0.5	0.10
Lipoprotein a *mg/dL*	16.5 ± 0.5	16 ± 0.6	0.23
Diabetes mellitus%	13.3	25.0	0.36
Hypertension%	40.0	57.5	0.22
Cigarette smoking%	40.0	47.5	0.70
Hyperlipidemia%	33.3	50.0	0.25
Percent of patients requiring at least 2 AVF%	60.0	0.0	0.0001
History of TIA%	13.3	0.0	0.0001
Ischemic cardiopathy%	23.3	0.0	0.0001
Amputation of a limb%	16.6	0.0	0.0001
Arterial thrombotic events%	100.0	0.0	0.0001
History of aspirin use%	16.6	0.0	0.0001

[a]By chi-square analysis for categorial variables (sex, diabetes, hypertension, smoking, hyperlipidemia, need of at least 2 AVF, TIA, ischemic cardiopathy, amputation of a limb, arterial thrombotic events, aspirin use); by ANOVA for continuous variables (age, dialytic age, hemoglobin, and lipoprotein a).

theory holds that aPL may induce a procoagulant activity in endothelial cells and monocytes.

Recently, aPL, specifically LAC, IgG, and IgM anticardiolipin antibodies (ACA-IgG and ACA-IgM), antiprothrombin antibodies, and anti-2-GPI antibodies, have been extensively studied in patients on MHD [15–18]. However, the role of these antibodies in the pathogenesis of thrombosis is still controversial [19–22] and further studies on their potential procoagulative role are indicated. In summary, the data suggest that vascular endothelial cell damage associated with thrombin formation is implicated in vascular occlusion in primary and secondary aPL syndrome.

The present study explores the alternative or additional role of a different class of antibodies, anti-protein C and anti-protein S, on vascular endothelial cell damage associated with hypercoagulability, in patients on MHD with and without thrombosis of vascular access.

METHODS

Patients and controls

A total of 70 patients on MHD for 24 to 36 months were enrolled for the study. Their characteristics are given in Table 1. Thirty patients presented thrombotic complications of the vascular access and other vascular locations, such as coronaries, retina, and inferior limbs, and were assigned to the TC group. Forty patients were without thrombotic complications and were termed the NTC group (Table 1).

All patients were dialyzed using an end-to-side vein-artery anastomosis of the cephalic vein and radial artery. Arteriovenous fistula (AVF) was monitored by Doppler ultrasound every 6 months for dysfunction (Table 2). Patients with alterations of the primary fistula, for example, stenosis of a portion of vascular access, were not included in the study. Absence of recirculation was assessed every 3 months by a conventional glucose test. All patients received conventional hemodialysis with bicarbonate bath and used polysulfone membranes. The average length of each dialysis was 4 hours, average blood flow was 300 to 350 mL/min, and the adequacy of dialysis was assessed on a monthly basis using the Kt/V, which was kept in the range 1.3 to 1.4. Heparin was used as anticoagulant in patients in all patients. Patients were studied along with 400 healthy age- and sex-matched blood donor volunteers recruited at our University Hospital.

Table 2. Characteristics of vascular access at beginning of the study

Type of vascular access	TC	NTC
Arteriovenous fistula	100%	100%
Time to first event of thrombosis		
After 3 months of dialysis	33.3%	0%
After 6 months of dialysis	13.3%	0%
After 12 months of dialysis	20%	0%
After 24 months of dialysis	33.3%	0%
Doppler analysis		
Sluggish flow	0%	0%
Stenosis	0%	0%
Recirculation		
Glucose test (positivity)	0%	0%

The causes of uremia in patients of the TC group were: polycystic kidney disease (PCKD) (4 cases), chronic pyelonephritis (14 cases), nephroangiosclerosis (4 cases), diabetic nephropathy (5 cases), glomerulonephritis of unknown origin (3 cases). In the NTC group causes for uremia were: PCKD (6 cases), chronic pyelonephritis (14 cases), nephroangiosclerosis (6 cases), diabetic nephropathy (7 cases), focal and segmental glomerulosclerosis (3 cases), and glomerulonephritis of unknown origin (4 cases). Hypertension, cigarette smoking, diabetes, and dyslipidemia are now equally distributed between groups. All patients, with the exception of those with PCKD, received erythropoietin.

There were significant differences ($P < 0.0001$) between TC and NTC patients: 60% of TC patients needed at least 2 AVF, one third had history of TIA, 23% of them had ischemic cardiopathy, 16.6% underwent amputation of a limb, 100% had arterial thrombotic events.

Laboratory investigations

All patients were tested for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fg), coagulation Factors of the intrinsic and extrinsic pathway, antithrombin III (AT-III), protein C (PC), protein S (PS), resistance to activated protein C (APC-resistance), prothrombin fragment 1+2 (F 1+2), plasminogen (PLG), tissue type plasminogen activator (tPA),

plasminogen activator inhibitor-1 (PAI-1), homocysteine (Hcy), C-reactive protein, anticardiolipin antibodies type M and G (ACA-IgM and ACA-IgG), anti-prothrombin antibodies type M and G (aPT IgM and IgG), anti-β_2 GPI, anti-protein C antibodies, and anti-protein S antibodies. Genetic analysis of Factor V Leiden mutation and prothrombin mutation were tested in all patients as very common cardiovascular risk factors.

Blood samples (4.5 mL) were collected in silicone-treated glass tubes by venipuncture, immediately before the midweek hemodialysis session. Blood samples in TC patients were collected 6 weeks after the acute ischemic event. In this respect, the hemostatic storm was definitively quenched. All patients and controls were asked to provide base-line EDTA anticoagulated samples of whole blood. Trisodium citrate (0.1 mol/L) in 1/10 volume ratio was added as anticoagulant. Citrated blood was centrifuged for 20 minutes at 1700*g* at 4°C. The supernatant was divided into aliquots and stored at −80°C.

Coagulation factors were determined with a one-stage clotting assay using commercially available reagents. Fibrinogen concentration was determined in an automated coagulation laboratory autoanalyzer (ACL 2000; Instrumentation Laboratory, Milan, Italy). Protein C (Berichrom Protein C, Dade Behring; Marburg, Germany) and S (Instrumentation Laboratory) plasma levels were estimated with a functional clotting assay. Tissue-type plasminogen activator and PAI-1 were assayed by enzyme immunoassay (Hyphen Biomed, Andresy, France). Prothrombin fragment 1+2 levels was measured by an enzyme-linked immunosorbent assay (ELISA) method (Enzygnost F1+2 micro; Dade Behring).

Anticardiolipin antibodies (ACA-IgG and IgM), anti-prothrombin antibodies (aPT-IgM and IgG), and anti-β_2GPI (Hyphen Biomed) were measured by ELISA using commercially available kits (Hyphen Biomed).

Anti-protein C and anti-protein S were measured by ELISA using an experimental kit (Hyphen Biomed).

Hcy concentration was determined according to Perna et al [23]. Briefly, the procedure involves a preliminary step of reduction and release from albumin, using tri-*n*-butyl-phosphine in dimethylformamide, followed by precolumn derivatization with ammonium 7-fluorobenzo-2-oxa-1, 3-diazole-4-sulphonate (SBD-F). Separation was accomplished on a C18, 5-μm, 250 × 4.6 mm, reverse phase column ("Luna; " Phenomenex, Torrance, CA, USA). The mobile phase was 0.1 mol/L KH_2PO_4, pH 2.1, containing 4% acetonitrile, with a flow rate of 1.0 mL/min. Micromolar concentrations of homocysteine are referred to 1 liter. Detection conditions were optimized for homocysteine. Fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm using a Shimadzu RF-535 fluorescence detector (Shimadzu Co., Kyoto, Japan), equipped with a Shimadzu Chromatopac C-R6A data processor (Shimadzu Co.).

C-reactive protein measurement was carried out according to an immunoenzymatic method (Roche Diagnostics, Milan, Italy).

Genomic DNA (200 ng) from peripheral white blood cells was extracted according to standard international procedures. Factor V G 1691A mutation and Factor II G20210A polymorphisms were searched using polymerase chain reaction (PCR) technique in a second laboratory, where the investigators were unaware of each subject's status as a case patient or control.

Table 3. Factor II, Factor V, Factor VII, Factor X, ATIII, protein C, protein S, plasminogen (PLG), APCr tPA, homocysteine, C-reactive protein, PAI-1, F1+2, and Factor VIII in TC, NTC, and controls

Variables	TC	NTC	Controls	*P*
FII%	90 ± 5	86 ± 12	100 ± 25	n.s.
FV%	76 ± 11	81 ± 7	80 ± 20	n.s.
FVII%	96 ± 11	94 ± 10	100 ± 25	n.s.
FX%	102 ± 14	93 ± 13	100 ± 25	n.s.
ATIII%	103 ± 4	109 ± 3	98 ± 15	n.s.
Protein C%	92 ± 4	91 ± 4	98 ± 15	n.s.
Functional protein S%	80 ± 4	93 ± 6	98 ± 15	n.s.
PLG *mg/dL*	87 ± 13	85 ± 4	135 ± 12	n.s.
APCr *N* ratio	1 ± 0.02	0.95 ± 0.01	>0.75	n.s.
tPA *ng/mL*	2 ± 0.7	2.3 ± 0.5	5 ± 0.5	n.s.
C-reactive protein	1.19 ± 0.19	1.02 ± 0.10	0.7 ± 0.10	$P = 0.001$
Homocysteine	27.8 ± 1.3	23.6 ± 2.07	7.98 ± 1.48	$P = 0.001$
PAI-1 *ng/mL*	49 ± 3.3	33 ± 3.4	22 ± 11.5	$P = 0.01$
F1+2 *ng/mL*	5.4 ± 1.3	4.5 ± 0.9	0.35 ± 0.35	$P = 0.006$
F VIII%	180 ± 25	122 ± 8	100 ± 2.75	$P = 0.007$

Statistical analysis

Statistical evaluation was performed using the Statistical Package for Social Science (SPSS 6.1 for Macintosh, Chicago, IL, USA). The analysis of variance (ANOVA) test or chi-square was used to determine the significance of differences between groups. Data were expressed as mean values ± standard deviation (SD) and as prevalence. Values of $P < 0.05$ were considered significant.

RESULTS

No significant difference was found between TC, NTC, and controls with respect to plasma Factor II, Factor V, Factor VII, Factor X, protein C, protein S, ATIII, PLG, APC-resistance and tPA (Table 3). Also, data on PT, PTT, and fibrinogen (not included) did not disclose statistical differences between TC and NTC patients. C-reactive protein was increased in TC and NTC patients in comparison with healthy controls ($P = 0.001$).

Hcy was increased significantly both in TC and NTC compared to the control group ($P = 0.001$) (Table 3).

Patients with TC showed a significant elevation of Factor VIII procoagulant concentration ($P = 0.007$), a

Table 4. Prevalence of anti-phospholipid antibodies in patients TC and NTC

Anti-phospholipid antibodies	Normal values	TC prevalence%	NTC prevalence%	*P* value[a]
anti-Protein S *U/mL*	<10	47%	8%	0.0001
anti-Protein C *U/mL*	<10	100%	6%	0.0001
ACA IgG *U/mL*	<7	84%	56%	0.115
ACA IgM *U/mL*	<5	64%	15%	0.001
aPT IgG *U/mL*	<7	34%	37%	0.789
aPT IgM *U/mL*	<7	14%	12%	0.055
anti-β2 GPI *U/mL*	<5	57%	32%	0.058

Significance for $P < .05$.
[a]Chi-square test.

Table 5. Absolute values of anti-phospholipid antibodies in patients TC, NTC, and controls

Anti-phospholipid antibodies	TC	NTC	Control	*P**
anti-Protein S *U/mL*	10.1 ± 2.8	6.85 ± 2.3	1.7 ± 0.5	0.0001
anti-Protein C *U/mL*	24.3 ± 8	7.23 ± 2.1	1.6 ± 0.5	0.0001
ACA IgG *U/mL*	13 ± 5.8	7.86 ± 3.6	2.63 ± 1.05	0.0001
ACA IgM *U/mL*	7.0 ± 3.6	3.2 ± 2.5	1.27 ± 0.29	0.001
aPT IgG *U/mL*	5.65 ± 2.63	6.79 ± 5.0	2.33 ± 0.88	0.04
aPT IgM *U/mL*	7.48 ± 2.64	4.83 ± 3.35	2.13 ± 0.77	0.04
anti-β2 GPI *U/mL*	5.51 ± 1.52	4.38 ± 1.1	1.7 ± 0.61	0.001

significant elevation of F1+2 plasma level ($P = 0.006$), and an increased PAI-1 level ($P = 0.01$) compared to NTC and the control group (Table 3). Factor VIII did not correlate significantly with CRP.

The antiphospholipid levels are reported in Table 4 and Table 5. Elevated anti-protein C (IgM) plasma levels are found in 100% of TC patients and in only 6% of NTC patients ($P = 0.0001$), with a significant difference with the ANOVA test ($P = 0.001$). Anti-protein S (IgG) plasma levels were elevated in 47% of TC patients and in 8% of NTC patients ($P = 0.0001$), with a significant difference with the ANOVA tests. Positivity for anti-β_2GPI was found in 57% of TC patients and 32% of NTC patients ($P = 0.056$), with a significant difference in mean ± SD plasma levels at ANOVA test (Table 5). The prevalence of ACA-IgG antibodies was 84% in TC and 56% in NTC ($P = 0.115$), while the prevalence of ACA IgM antibodies was 64% in TC and 15% in NTC, respectively ($P = 0.001$), with a significant difference for ANOVA test only for ACA IgM plasma levels ($P = 0.001$). No differences in the prevalence of aPT-IgG and aPT-IgM antibodies were found between TC and NTC patients, while a significant difference was found at ANOVA test considering the different group of subjects for aPT-IgG and aPT-IgM ($P = 0.04$).

Genetic analysis shows that Factor V Leiden mutation was found in 1 TC patient and in none of the patients of the NTC group. One patient over 70 represents 1.4% of all patients. The prothrombin mutation was not found in both groups. In the general population Factor V mutation is found in about 2% of healthy subjects, while prothrombin polymorphism heterozygosity is found in 4%.

DISCUSSION

Anti-protein C and anti-protein S antibodies were measured for the first time in uremic patients on maintenance hemodialysis. All available knowledge in the field has been obtained either in patients with systemic lupus erythematosus (SLE) or in patients without SLE but with venous thromboembolism [24–27]. The data demonstrate a significantly greater prevalence of anti-protein C antibodies and anti-protein S antibodies in hemodialyzed patient on MHD with thrombosis of vascular access (TC), a novel finding indicating that in uremia, in addition to endothelial damage [28, 29], other pathogenetic mechanisms may cause hypercoagulability.

In order to understand the importance of present results, it might be instrumental to assign a proper role to each of the factors studied by including them in a possible model of hemostatic derangement in uremia. PAI-1 is a molecule of endothelial origin and is overproduced in endothelial dysfunctions, such as in uremia. Patients with thrombotic complications show a significant increase in PAI-1, FVIII, F1+2 compared to controls, as well as to patients without thrombotic complications. The excess PAI-1 released in the bloodstream inhibits tPA and blocks the fibrinolysis system, causing hypofibrinolysis (i.e., low efficiency thrombolysis). Despite hypofibrinolysis, an increase of D-dimers was observed, but it should be stressed that their increase is lower than expected, considering that the coagulation pathway is activated in excess (Fig. 1).

F1+2, an indicator of abnormal activation of the coagulation pathway, is a molecule released during the activation of prothrombin to thrombin. Increased levels of F1+2 could be related to the presence of anti-prothrombin antibodies (Abs) and anti-β2 GP-I Abs. In fact, anti-prothrombin blocks the prothrombinase complex in a status of activation. This mechanism continuously stimulates the transformation of prothrombin to thrombin and the consequent release of F1+2 (Fig. 1).

In turn, anti-β2 GP-I antibodies compete with Factor V in binding β2 GP-I. Therefore, when β2 GP-I is bound to the antibody, it is no longer available to bind Factor V and present it to the protein C system, which is the pathway that inactivates activated Factor V [30]. Factor V (not inactivated) forms the prothrombinase complex and increases the formation of thrombin and F1+2 (Fig. 1).

Increased Factor VIII causes activation of the coagulation pathway because Factor VIII (Fig. 1) is an important cofactor in the activation of Factor X by the tenase complex. Elevated Factor VIII could be potentially prothrombotic either by increasing the stability of the tenase complex or by conferring a relative resistance

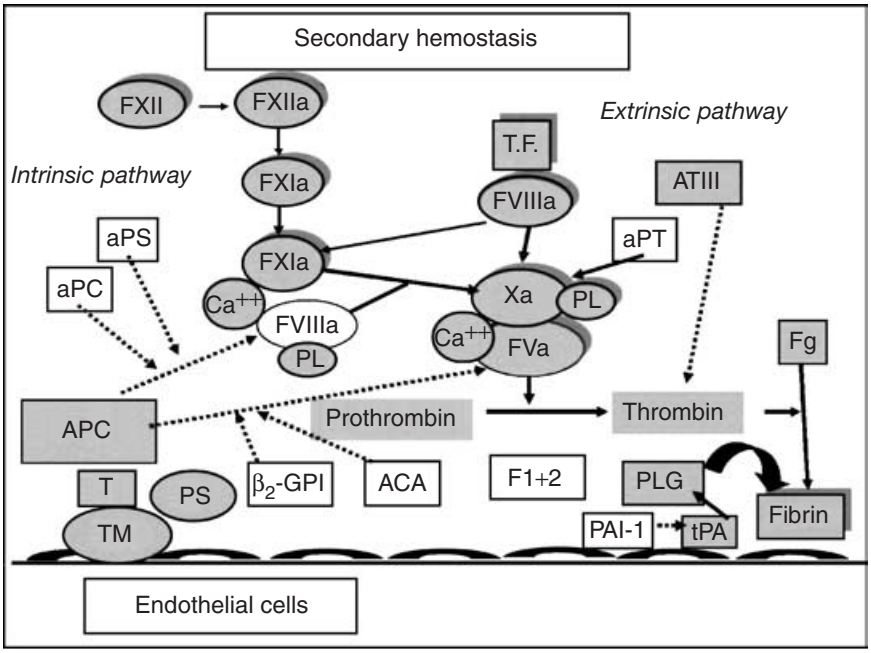

Fig. 1. Synopsis of all intrinsic and extrinsic pathways of secondary hemostasis that lead to thrombin formation and fibrin deposition. White squares and circles indicate derangements in patients on maintenance hemodialysis. Broken lines indicate inhibition.

to APC degradation. Elevated levels of Factor VIII were associated with venous thrombosis in the Leiden Thrombophilia Study, and the association has also been observed subsequently [31].

It is well known that an increase in Factor VIII is due to endothelial damage as well to the presence of the anti-protein C antibodies. Elevations of Factor VIII in the present study were not associated with the inflammatory state (PCR vs. FVIII, $R = 0.112$, $P = 0.742$).

Hcy levels produce interactions with the anticoagulant pathway, as well as with the fibrinolytic system and the endothelial functions. These interactions lead to inhibition of thrombomodulin-dependent activated protein C system, which, in turn, leads to persistent thrombin activation and formation. The activated protein C system also interferes with tPA endothelial release into the vasculature, thus predisposing to hypofibrinolysis. Finally, high Hcy plasma levels may interfere with subendothelial cell proliferation via metalloproteinase inducible gene activation such as MPP-9 subtype. Biologically plausible mechanisms of vascular damage have been suggested, including effects on endothelium, platelet functions, coagulation factors, and lipoprotein oxidation. Interactions between Hcy plasma levels and conventional risk factors may have implications for risk management and for our understanding of the causes of vascular access thrombosis [32, 33].

In order to understand the possible role for anti-protein C and anti-protein S antibodies in the hemostatic derangement in uremia, one should start from the fact that anti-protein C antibodies induce hypercoagulability by inhibiting the protein C system, and by blocking the enzymatic inactivation of Factor VIII, resulting in an increase of activated Factor VIII. This, in turn, stimulates the formation of thrombin, which, after saturating all the thrombomodulin binding sites, is diverted to the formation of fibrin. The thrombin excess increases the procoagulation pathway rather than showing the anticoagulation effect of binding with thrombomodulin.

Activated protein C acts as an anticoagulant by cleaving multiple bonds and thereby destroying the membrane-bound activated forms of coagulation Factors V (Va) and VIII (VIIIa). This reaction is accelerated by protein S acting as a cofactor. Like protein C, protein S is a glycoprotein that undergoes vitamin K–dependent post-translational carboxylations to form gamma-carboxyglutamic acid ("Gla") residues that allow its binding to negatively charged phospholipid surfaces. Protein S acts as a cofactor by increasing the affinity of activated protein C for phospholipids in the formation of the membrane-bound protein C-ase complex.

Anti-protein S antibodies target protein S, which is cofactor of protein Cact. We can hypothesize that anti-protein S antibodies induce hypercoagulability by inhibiting the protein C system and by blocking the enzymatic inactivation of Factor VIII, resulting in an increase of activated Factor VIII (Fig. 1).

The present data do not assign a role to Factor V and prothrombin mutations; however, further investigation is warranted to define the role of genetic analysis in vascular access thrombosis.

For the sake of comparison, the above data are presented in Table 6 along with data from some very relevant papers in the field. In the table, one can appreciate the

Table 6. Summary of principal studies on coagulation in hemodialysis patients

Authors	Year of publication	Number of patients	Vascular access	Values of PAI-1 *ng/mL*	Values of F1+2 *nmol/L*	Values of FVIII %	Values of Hcy *µmol/L*	Prevalence of aPC-abs	Prevalence of aPS-abs	Prevalence of ACA IgG-abs	Prevalence of β2-GPI-abs
Vaziri ND et al	1994	31 patients (17 men and 14 women)	Not indicated	Not studied	Not studied	108 ± 9	Not studied	Not studied	Not studied	Not studied	Not studied
Prakash R et al	1995	17 patients	Arteriovenous fistula (AVF)	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied	6%	Not studied
				57.5 ± 33.6 FD	3.09 ± 0.96 FD						
De Marchi S et al	1996	30 patients (18 men and 12 women)	AVF	24.7 ± 21.1 WFD	3.14 ± 0.87 WFD	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied
Manns BJ et al	1999	89 patients	AVF	Not studied	Not studied	Not studied	29.8	Not studied	Not studied	Yes	Not studied
Segarra A et al	2001	200 patients (120 men and 80 women)	Not indicated	35.4 ± 7.2	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied
Palomo I et al	2002	208 patients (96 men and 112 women)	AVF	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied	6.7%	4.3%
				49 ± 3.3 TC	5.4 ± 1.3 TC	180 ± 25 TC	27.8 ± 1.3 TC	100% TC	47% TC	84% TC	57% TC
Present data		70 patients	AVF	33 ± 3.4 NTC	4.5 ± 0.9 NTC	122 ± 8 NTC	23.6 ± 1.5 NTC	6% NTC	8% NTC	56% NTC	32% NTC

role of our data and the time course of advancement in the field. It is evident that previous studies on coagulation in uremia have mainly focused their attention on differences in coagulation cascade, and/or on anticardiolipin antibodies and lupus anticoagulant, without screening for different subclasses of autoantibodies against phospholipid, endothelial, or platelet antigens.

CONCLUSION

We conclude that thrombotic complications in uremic patients are the result of changes in the secondary hemostasis and in fibrinolysis, differentiating them from hemorrhagic complications due to changes in primary hemostasis. The alterations of secondary hemostasis and fibrinolysis are due to endothelial damage and to the additive effects of some classes of antiphospholipid antibodies. These changes, for unknown reasons, are more severe in some patients, who are consequently more prone to thrombotic complications. Studies on the antigens of vascular endothelium to which autoantibodies bind might provide a further insight into their pathogenicity for thrombosis in patients on MHD.

ACKNOWLEDGMENTS

The study was supported by Italian Space Agency (ASI), the Ministry for University and Research, and by the University of Naples. The authors wish to express their warmest thanks to Molly Fudge Mastrangelo (Ph.D.) and to Giovanni Mastrangelo (M.D.), Cumberland (Maryland-USA), for their critical review of the manuscript.

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